Establishing how Bacterial Cells Position the Division Site

Christopher Daniel Andrade Rodrigues

A thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy

January 2011

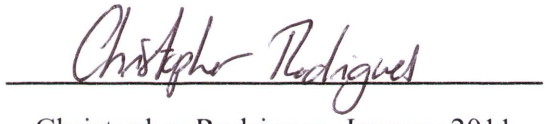
The iThree Insitute
University of Technology, Sydney NSW,
Australia

Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the written preparation of the thesis, and all experimental work associated with it has been carried out solely by me, unless otherwise indicated.

Finally, I certify that all information sources and literature used are acknowledged in the text.



Christopher Rodrigues, January 2011

Acknowledgements

First and foremost, I wish to thank my excellent supervisor, Professor Liz Harry. I thank her for giving me an interesting and exciting project and for being a very encouraging and understanding supervisor. Thank you for believing in me Liz! I will always remember the intellectually stimulating meetings in her office discussing research ideas and models. Liz has taught me so many things about how to do science and also how to live a balanced life - I will always remember the time we spent in Mexico, sailing and snorkelling around the reefs of the Caribbean. I finally wish to thank her for the precious time and effort spent on reading and commenting on this thesis.

I wish to thank the members, past and present, of the Harry lab that have always provided assistance, interesting discussions and entertaining social events, including the karaoke and Yum Cha lunches! Thank you goes to Adeline Quay, Kylie Turner, Leigh Monahan, Rowena Lock, Patricia Quach, Jo Packer, Torsten Theis, Rebecca Rashid, Phoebe Peters, Michael Strauss, Andrew Liew, Michelle Tu and Sinead Blaber. A special thank you goes to Janniche Torsvik for being a fun and silly friend to be around. Finally, I thank Joana Santos for being my PhD sister and a good friend.

I particularly wish to thank Professor Gerry Wake and Dr. Rebecca Rashid, Rebecca for mentoring me during the first year of my PhD, and Gerry for being very interested in my project and for being a critical commentator on most aspects of my work. I also wish to thank Jaye Lu and Isabella Hajduk, two great summer students that participated in my work. It was great to see these two young ladies become junior scientists. I also thank Isabella Hajduk and Sinead Blaber for proof-reading this thesis.

I would also like to thank Prof. Jeff Errington and Prof. Alan Grossman for providing bacterial strains and Dr. Fraser Torpy for advice on statistics.

On a personal note I would like to thank my partner, Thomas, for encouraging and believing in me and for being there all steps of the way. A special thank you goes also

to my parents, Ana and Francisco, and my sister Sabrina for their encouragement and support.

Lastly, I would like to acknowledge the financial assistance provided by the Australian Government, in the form of an Australian Postgraduate Award.

Contents

Table of Figures and Tables

Figures

Tables

Publications

Journal article

S. Moriya^, R. Rashid^, **C.D. Andrade Rodrigues**^ and E.J. Harry (2010) Influence of the nucleoid and the early stages of DNA replication on positioning the division site in *Bacillus subtilis*. *Molecular Microbiology* 76: 634-47

^First author with equal contribution

Conference proceedings[a]

C.D. Andrade Rodrigues and E. J. Harry - November, 2010 - Bacterial Cell Biology Meeting - Cancun, Mexico - **Oral presentation** - "The Min system and nucleoid occlusion do not identify the division site in *Bacillus subtilis*; they regulate its utilization"

C.D. Andrade Rodrigues and E. J. Harry - July, 2010 - Australian Society for Microbiology Meeting- Sydney, Australia - **Poster Presentation** - "How bacteria identify their middle: challenging a paradigm"

C.D. Andrade Rodrigues, S. Moriya, R. Rashid, and E. J. Harry - September, 2009 – Gram-Positive Bacteria Meeting - Kobe, Japan - **Invited speaker** -"Evidence of a Noc-independent mechanism linking DNA replication to cell division in *Bacillus subtilis*"

S.Moriya, R. Rashid, C.D. Andrade Rodrigues, E.J. Harry - August, 2009 - EMBO Workshop "Frontiers of Prokaryotic Cell Biology" - Oxford, UK - **Oral presentation** - "A new model for positioning of the cytokinetic Z ring: initiation of replication potentiates the division site."

C.D. Andrade Rodrigues, S. Moriya, R. Rashid E. J. Harry - July, 2009 - American society for Microbiology, Prokaryotic Development Conference - Boston, USA - **Poster presentation** - "Evidence of a Noc-independent mechanism linking DNA replication to cell division in *Bacillus subtilis*"

C.D. Andrade Rodrigues, S. Moriya, R. Rashid, and E. J. Harry - August, 2008 - Molecular Genetics of Bacteria and Phages - Cold Spring Harbour, New York, USA - **Poster Presentation** " A Link between DNA Replication and Cell division in *Bacillus subtilis*"

C. D. Andrade Rodrigues, S. Moriya, R. Rashid, and E. J. Harry - July, 2008 -

Australian Society for Microbiology Meeting - Melbourne, Australia - **Oral presentation** - "A Link between DNA Replication and Cell Division in Bacteria"

R. Rashid, C. D. Andrade Rodrigues, S. Moriya, E. J. Harry - April, 2008 – 162nd Meeting of the Society for General Microbiology - Edinburgh, UK - **Oral presentation** - "Relationship between chromosome structure and Z ring placement"

C. D. Andrade Rodrigues, S. Moriya, R. Rashid, and E. J. Harry – November, 2007 - RNSH/UTS/USyd/Kolling Institute XXIVth Annual Scientific Research Meeting - Sydney, Australia - **Poster Presentation** - "Coordinating DNA replication with Bacterial cell division"

R. Rashid, S. Moriya, C. D. Andrade Rodrigues and E. J. Harry – July, 2007 - Australian Society for Microbiology Meeting - Melbourne, Australia - **Poster presentation** - "Coordination between proper Z ring placement and DNA replication in *B. subtilis*"

[a] The presenting author is underlined.

Abbreviations

A	adenine
Aa	amino acid
Ab	antibody
AGRF	Australian Research Genome Facility
ATM	atomic force microscopy
B.	*Bacillus*
β	beta
bp	base pair(s)
BP	band pass
BSA	bovine serum albumin
C	cytosine
cat	chloramphenicol resistance gene
CCD	charged coupled device
DAPI	4'6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
dsDNA	double stranded deoxyribonucleic acid
E.	*Escherichia*
ECT	electron cryotomography
ECL	enhanced chemiluminescence
et al.	and others
ermC	erythromycin resistance gene
FRAP	fluorescence recovery after photobleaching
fts	filamentation temperature-sensitive
G	guanine
g	centrifugal force
g	gram (s)
GFP	green fluorescent protein
GMD	germination medium defined
h	hour(s)
HPUra	6-(-p-hydroxyphenylazo)-uracil

IFM	immunofluorescence microscopy
Ig	Immunoglobulin
IPTG	isopropyl-1-thio-β-D-galactopyranoside
kD	kilo Dalton(s)
L	litre(s)
LP	long pass
m	milli (10^{-3})
M	moles per litre
min	minute(s)
MQW	Milli-Q purified water
MSA	mineral salts A
MTS	membrane targeting sequence
n	nano (10^{-9})
NA	numerical aperture
N/A	not applicable
NBS	Noc-binding sites
neo	neomycin resistance gene
OD_x	optical density at (x refers to the wavelength in nm)
p	probability
P_{spac}	IPTG-inducible promoter
P_{spachy}	IPTG-hyper-inducible promoter
P_{xyl}	xylose-inducible promoter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDS	potential division sites
pH	power of Hydrogen
phleo	phleomycin resistance gene
RNase	ribonuclease A
ROR	round of replication
ROW	reverse osmosis purified water
rpm	revolutions per minute

RT	room temperature
S.	*Streptomyces*
sec	second(s)
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SMC	structural maintenance of chromosome
SMM	spizizen minimal medium
spp.	species
spc	spectinomycin resistance gene
T	thymine
TBAB	tryptose blood agar base
tet	tetracycline resistance gene
thy	thymine auxotroph
Tris	tris(hydroxymethyl)methylamine
Trp	L-Tryptophan
ts	temperature sensitive
U	units (enzyme activity)
UV	ultraviolet
V	volt(s)
v/v	volume per volume
W	watt
w/v	weight per volume
YFP	yellow fluorescent protein
μ	micro- (10^{-6})

Abstract

In virtually all bacteria cell division is essential and tightly regulated both temporally and spatially to ensure that cells divide precisely at the centre between segregated chromosomes. Failure to do so can lead to cell death. The earliest event in bacterial cell division is the polymerization of the highly conserved tubulin-like protein, FtsZ, to form a contractile structure called the Z ring, on the inner side of the cytoplasmic membrane at midcell and between chromosomes. The Z ring subsequently contracts causing the cell envelope to invaginate, generating two newborn cells. Thus the Z ring defines the position of the division site in bacterial cells.

How the Z ring is positioned precisely at midcell is a controversial topic that remains unresolved. Division site positioning has long been believed to occur via the combined action of two factors: the Min system and nucleoid occlusion. Both factors have been proposed to prevent Z ring assembly along the length of the cell, allowing it to assemble only once chromosomes segregate and nucleoid occlusion is relieved specifically at midcell. The research described in this thesis challenges this paradigm, providing compelling evidence that other mechanisms in addition to nucleoid occlusion and the Min system act to position the Z ring at midcell in *B. subtilis*. Moreover, this work also shows that nucleoid occlusion and the Min system do not define the Z ring position at midcell but rather ensure that the midcell division site is utilized efficiently.

A clue to an additional mechanism for positioning the Z ring has emerged from studies investigating the relationship between chromosome replication and Z ring position. The nature of this relationship has remained obscure for years. Part of this thesis involves a closer examination of this relationship. It was found that the ability to position the Z ring at midcell is linked specifically to the progress of the initiation stage of DNA replication, such that the frequency of Z rings at midcell increases as this stage of DNA replication is progressively completed. Moreover, this link was found to be nucleoid occlusion independent.

Spatial and temporal control of Z ring assembly has been widely attributed to the Min system and nucleoid occlusion. While inactivating both systems substantially affects

cell division, it is currently unknown whether their absence affects precise midcell Z ring positioning. This thesis deals with this question, and it was found that the combined effect of MinCD and Noc proteins actually affects the timing and efficiency of Z ring assembly, but not its spatial precision between nucleoids at midcell.

If Noc and MinCD proteins do not position the Z ring at midcell, what other factores may play this role? Two hypotheses were proposed to help explain the precise Z ring positioning observed in absence of *noc* and *minCD*: 1) Noc-independent nucleoid occlusion or 2) factors completely independent of nucleoid occlusion position the Z ring at midcell. Experiments designed to discriminate between these hypotheses showed that they are actually both valid: while the data obtained suggests that factors completely independent of nucleoid occlusion (Noc inclusive) and the Min system position the Z ring at midcell, it also suggested that other Noc-independent nucleoid occlusion factors prevent the Z ring from assembling at midcell over unreplicated DNA.